

Fig. 1

Figure 2

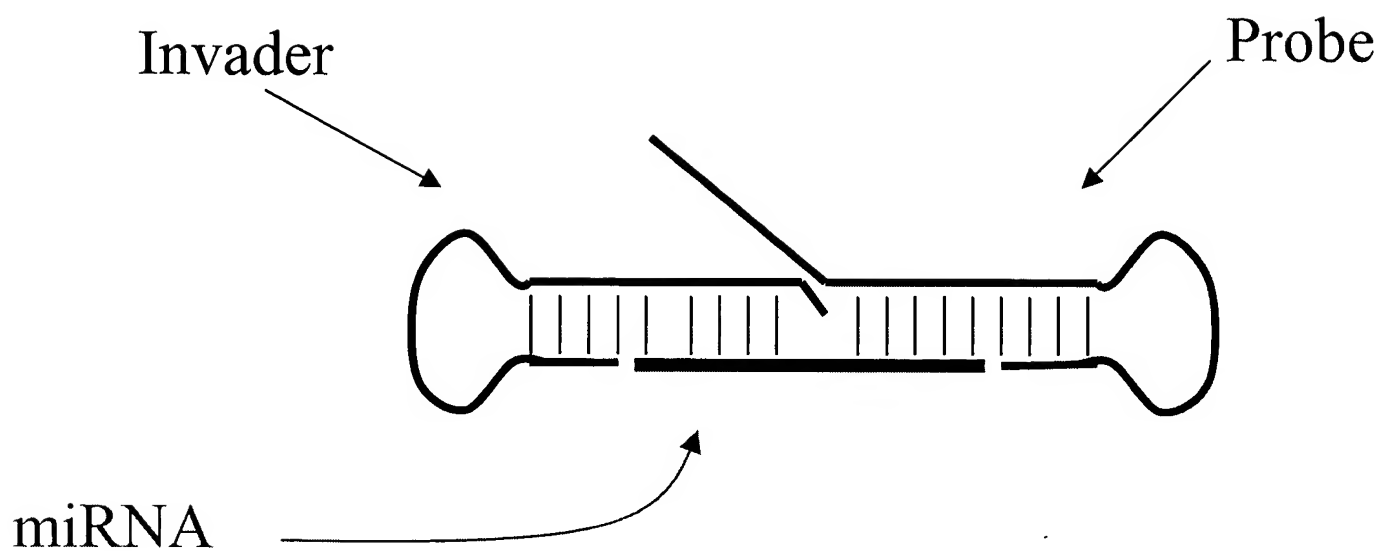


Figure 3

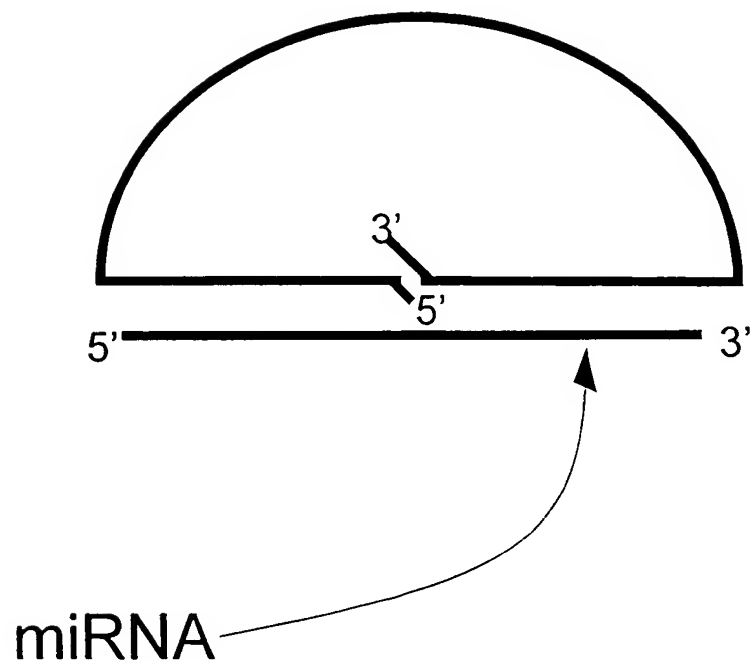
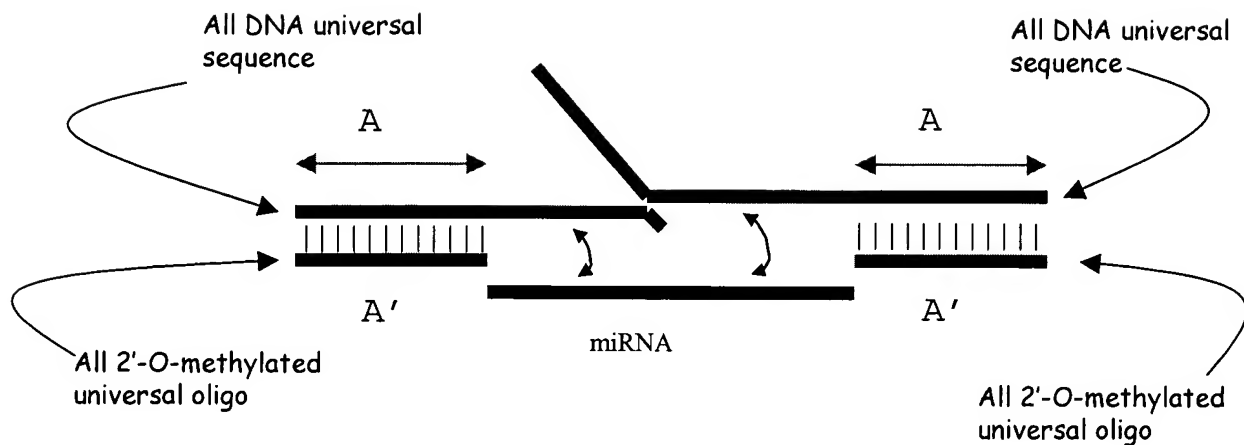


Figure 4



A = Universal sequence that is added to the 3' and 5' of probes and INVADER oligonucleotides, respectively.

From 5' to 3', the probe is composed of the 5'-flap, the miRNA complementary region, and the DNA universal sequence "A".

The INVADER oligonucleotide from 5' to 3', is composed of the DNA universal sequence "A" and an miRNA complementary region.

A' = 2'-O-methyl universal oligonucleotide that compliments the sequence "A" and is added to kits as a standard oligonucleotide.

FIG. 5

[illegible]

FIG 5

FIG. 5

SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:39	human miR-16	miRNA	uagcagcacgtaaauuugcg
SEQ ID NO:40	SRT	Secondary Reaction template	CCAGGAAGCAAGTGGAGGGGTGACggu
SEQ ID NO:41	human GAPDH	Invader oligo	ggaucauuuGGAACATGTAAACCATC
SEQ ID NO:42	human GAPDH	probe oligo	CCGCCGAGATCACGTAGTTGAGGTC-NH2
SEQ ID NO:43	human GAPDH	arrestor oligo	gaccucaacuacugauc
SEQ ID NO:44	human miR-125b	miRNA	uuccugagaccuuuacuuugga
SEQ ID NO:45	U6 RNA	Invader oligo	GGCCATGCTAATCTTCA
SEQ ID NO:46	U6 RNA	probe oligo	CCGCCGAGATCACTCTGTATCGTTTC-NH2
SEQ ID NO:47	U6 RNA	arrestor oligo	gaacgauacagagugauc
SEQ ID NO:48	RED FRET		Yct-cXt-tct-cag-tgc-g
SEQ ID NO:49	SRT	Secondary Reaction template	CCAGCAAGCAAGTGGTGTCTCGGGCggu
SEQ ID NO:50	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACTCTCA-NH2
SEQ ID NO:51	human let-7a miRNA	Invader oligo	AACTATACAACT
SEQ ID NO:52	human let-7a miRNA	probe oligo	CCGTCGCTGCGTTACTACCTCA-NH2
SEQ ID NO:53	human let-7a miRNA	Invader oligo	AACTATACAAACCG
SEQ ID NO:54	human let-7a miRNA	arrestor oligo	ugagguaguagacgcag
SEQ ID NO:55	human miR-15	probe oligo	AACGAGGCGCACATGTCTGTCTACgagccuuuuggcug
SEQ ID NO:56	human miR-15	Invader oligo	ggcagccuuuugcgcCACAACCATTC
SEQ ID NO:57	human miR-15	arrestor oligo	cgagccaaaggguagcagcacaugcg
SEQ ID NO:58	human miR-15	probe oligo	AACGAGGCGCACATGTCTGTCTCGCCACGCCG-NH2
SEQ ID NO:59	human miR-15	Invader oligo	GCTCGCCACGCCCGCACAAACCATTC
SEQ ID NO:60	human miR-15	stacker oligo	cggcguggcgagc
SEQ ID NO:61	human miR-15	arrestor oligo	cggcguggcgagcuagcagcacaugcg
SEQ ID NO:62	human miR-15	miRNA	uagcagcacauuauuguuug
SEQ ID NO:63	human miR-135	probe oligo	AACGAGGCGCACAAATAAAAGCCATAGCTCGCCACGCCG-NH2
SEQ ID NO:64	human miR-135	Invader oligo	GCTCGCCACGCCCGTTCACATAGGC
SEQ ID NO:65	human miR-135	arrestor oligo	cggcguggcgagcuuugccuuuuuauugcg
SEQ ID NO:66	human miR-15	arrestor oligo	uagcagcacaugcg
SEQ ID NO:67	human miR-15	probe oligo	AACGAGGCGCACATGTCTGTCTAGGCGAAGCC
SEQ ID NO:68	human miR-15	Invader oligo	GGCGAAGCCCGCACAAACCATTC
SEQ ID NO:69	human miR-15	probe oligo	AACGAGGCGCACATGTCTGTCTAGGCGAAg
SEQ ID NO:70	human miR-15	Invader oligo	ggcGAAGCCCGCACAAACCATTC
SEQ ID NO:71	human miR-15	probe oligo	AACGAGGCGCACATGTCTGTCTAGgcuugcc
SEQ ID NO:72	human miR-15	Invader oligo	ggcuugccCGCACAAACCATTC
SEQ ID NO:73	human let-7a miRNA	Invader oligo	GGCACTTTGTGCCAACTATACAAC
SEQ ID NO:74	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACTACGACGTTTTCGTCG
SEQ ID NO:75	human let-7a miRNA	Invader oligo	ggcacTTTGTGCCAACTATACAAC
SEQ ID NO:76	human let-7a miRNA	probe oligo	CCGTCGCTGCGTCTACTACTACGACGTTTTCgucg

FIG 5

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FIG. 5

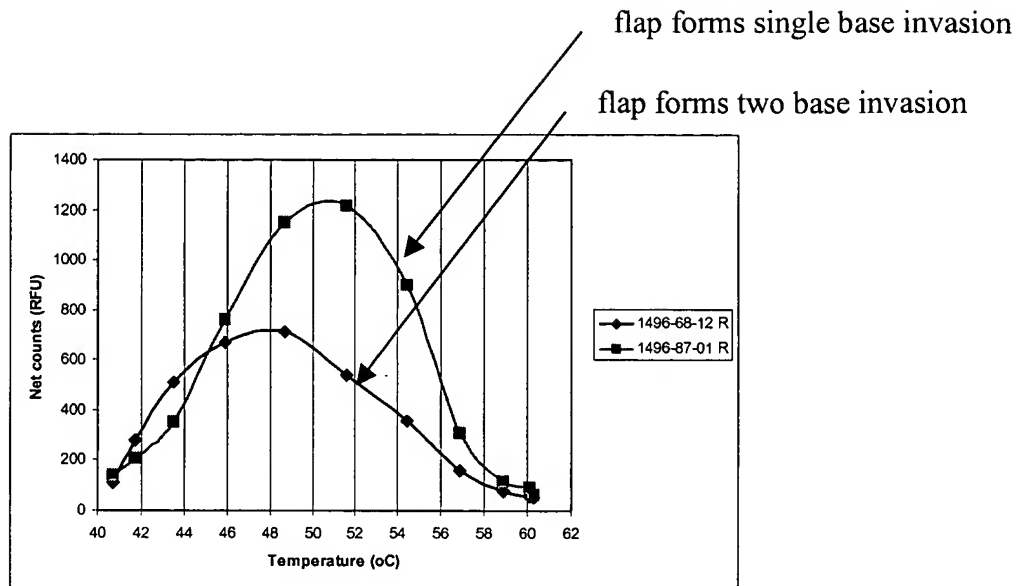
SEQ ID NO	Target	Oligo type	Sequence (5'-3')
SEQ ID NO:77	human miR-16 miRNA	Invader oligo	ggcagcuuuugcugccCGCCAATATTG
SEQ ID NO:78	human miR-16 miRNA	probe oligo	AACGAGGGCGCACTACGTGCTGCTACgagccuuuuggcucg
SEQ ID NO:79	human miR-16 miRNA	arrestor oligo	cgagccaaaaggcucgagcagcagcuagugcgc
SEQ ID NO:80	human miR-125b miRNA	Invader oligo	ggcagcuuuugcgtgctCACAAGTTAGA
SEQ ID NO:81	human miR-125b miRNA	probe oligo	AACGAGGGCGCACGGTCTCAGGGACgagccuuuuggcucg
SEQ ID NO:82	human miR-125b miRNA	arrestor oligo	cgagccaaaaggcucgcccugagagccgugcgc
SEQ ID NO:83	human let-7a miRNA	probe oligo	CCGTGCTGCGTCTACTACCTACgagcuuuuucgucgu
SEQ ID NO:84	human let-7a miRNA	Invader oligo	uggcacuuuuugccAACTATACAACCT
SEQ ID NO:85	human let-7a miRNA	probe oligo	CCGTGCTGCGTCTACTACCTACgagcuuuuucguc
SEQ ID NO:86	human let-7a miRNA	Invader oligo	gcacuuuuugccAACTATACAACCT
SEQ ID NO:87	precursor human let-7a	miRNA	gggcuuuuuggggagguagguuuuuaguuuuuaggaauuaccaccgguagaauu
SEQ ID NO:88	miR-124a 21nt	miRNA	gcauuuuuuuacccuuuuccugaaguccc
SEQ ID NO:89	miR-124a 22nt	miRNA	uaaggcacgcgguagaugcca
SEQ ID NO:90	miR-124a miRNA	probe oligo	uuuaggcacgcgguagaugcca
SEQ ID NO:91	miR-124a miRNA	arrestor oligo	CCGTGCTGCGTGGCTGCGTTCgagccuuuuggcucg
SEQ ID NO:92	miR-124a miRNA	Invader oligo	uaaggcacgcgagcag
SEQ ID NO:93	U6 RNA	probe oligo	ggcagcuuuugcugccTGGCATTACACA
SEQ ID NO:94	U6 RNA	Invader oligo	CCGCCGAGATCACCTAATCTTCTGTAT-NH2
SEQ ID NO:95	U6 RNA	arrestor oligo	CATCCTTGGCGCAGGGGCCATGA
SEQ ID NO:96	human miR-135	miRNA	auacagagaagauuaggugauc
SEQ ID NO:97	human miR-1d	miRNA	uaugcuuuuuuuuuccuaugugaa
SEQ ID NO:98	human miR-1d	probe oligo	uggauguaaagaagaauuuuuu
SEQ ID NO:99	human miR-1d	Invader oligo	AACGAGGGCGCACTTACATTCACgagccuuuuggcucg
SEQ ID NO:100	human miR-1d	arrestor oligo	ggcagcuuuugcugccATACATACTTCC
SEQ ID NO:101	human beta actin siRNA	probe oligo-antisense	cgagccaaaaggcucguggauguaaagugcgc
SEQ ID NO:102	human beta actin siRNA	Invader oligo-antisense	AACGAGGGCGCACAAAGATCATTCgguuucggcc
SEQ ID NO:103	human beta actin siRNA	arrestor oligo-antisense	ggcuucggccAATGAAGATCC
SEQ ID NO:104	human beta actin siRNA	probe oligo-sense	gcaugauuuuugcgc
SEQ ID NO:105	human beta actin siRNA	Invader oligo-sense	AACGAGGGCGCACCTTGATCTTCAGgcuucggcc
SEQ ID NO:106	human beta actin siRNA	arrestor oligo-sense	ggcuucggccAAGCAATGATA
			ugaagaucuaaggugcgc

FIG 5

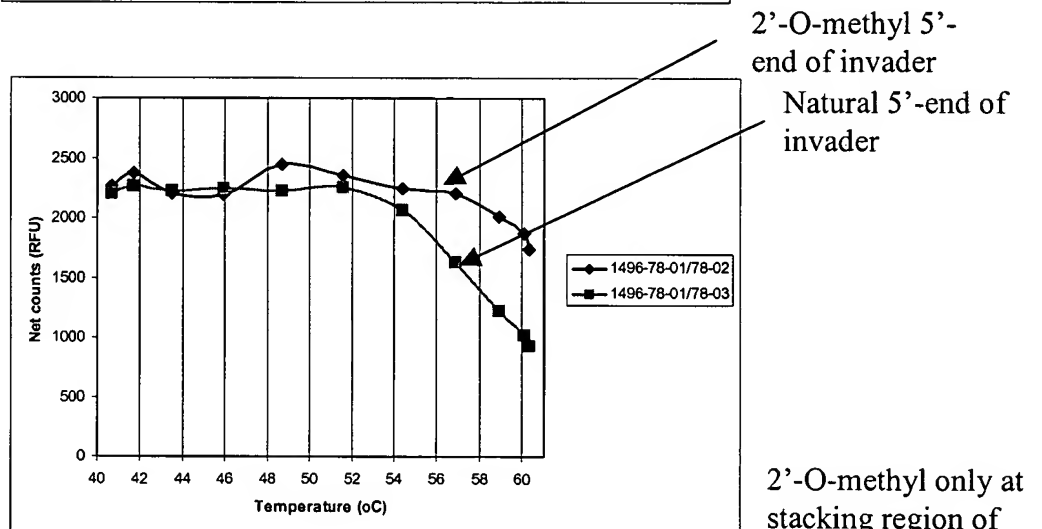
Figure 6

Design Optimization

1496-87-01R



1496-78-01 R



1496-96-01 R

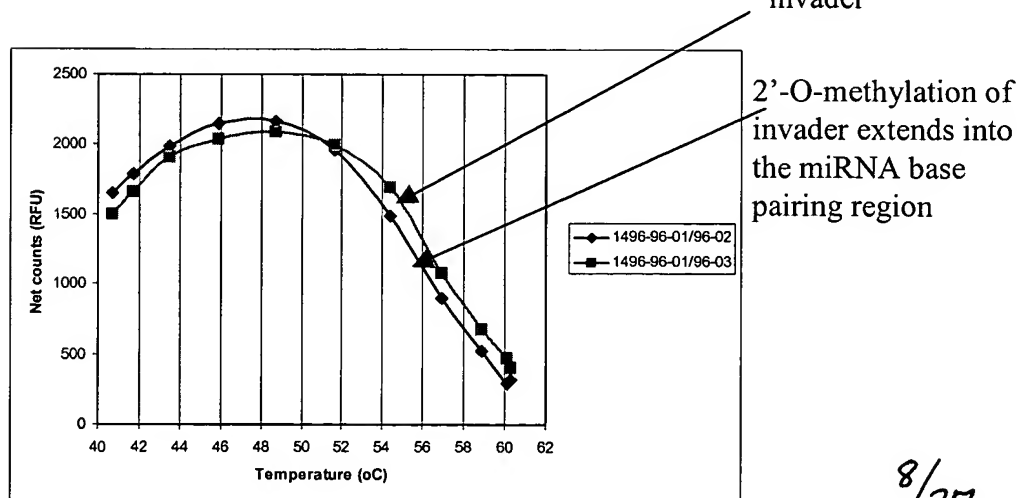


Figure 7
Design Optimization

Natural DNA stacking Invader

2'-O-methyl stacking invader

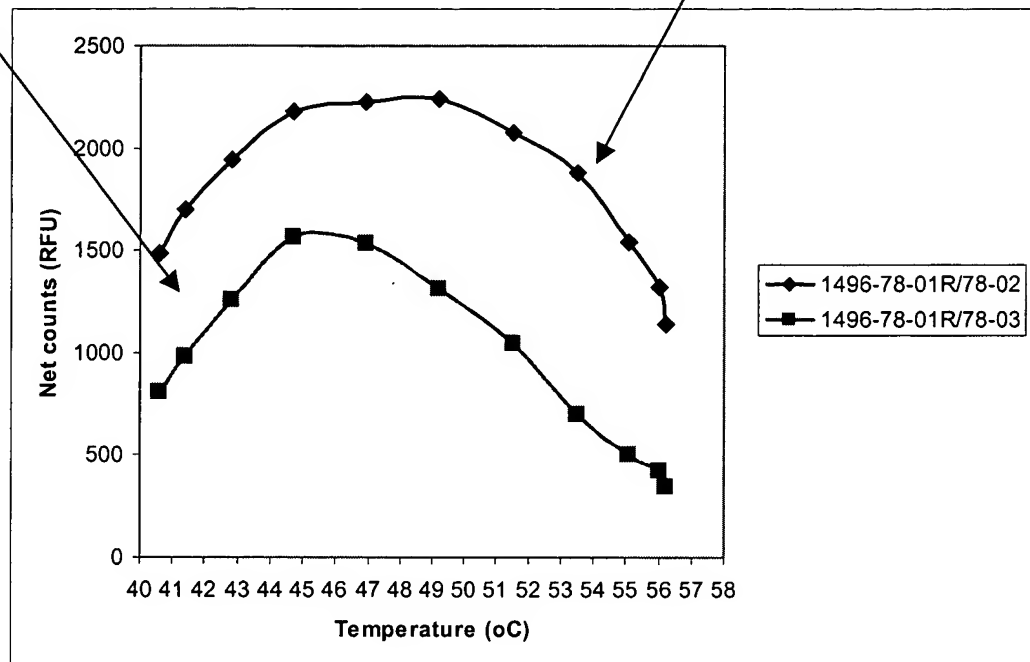
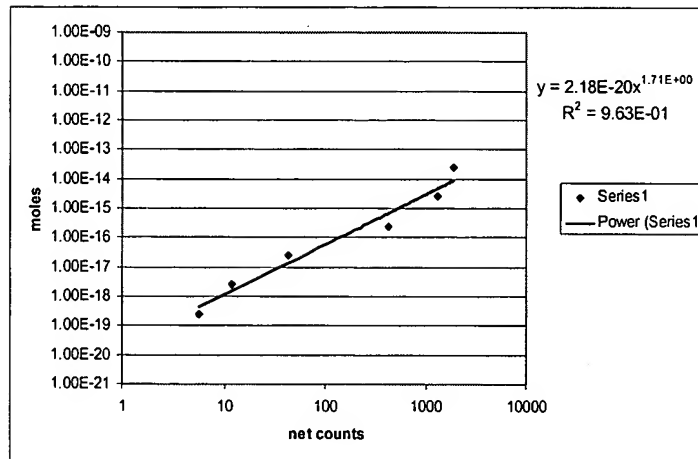
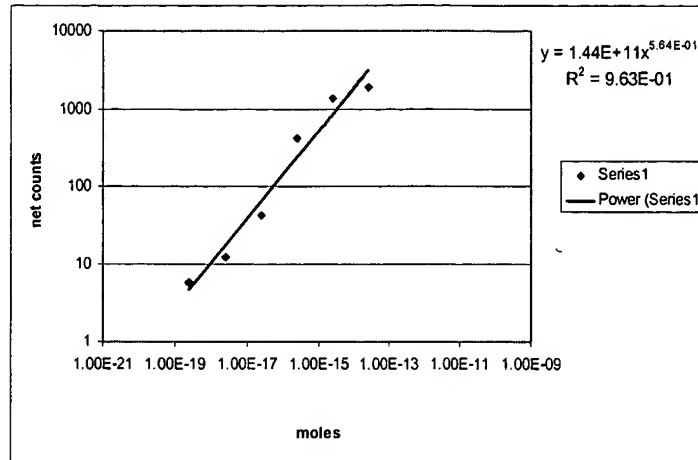
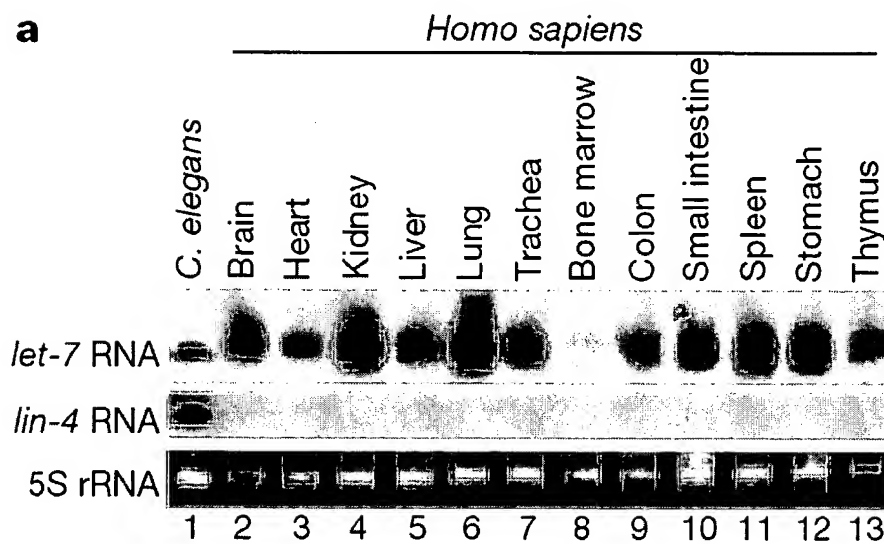


Figure 8
LOD let-7 (1496-78-01R)



a



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Figure 9
cross reactivity let-7

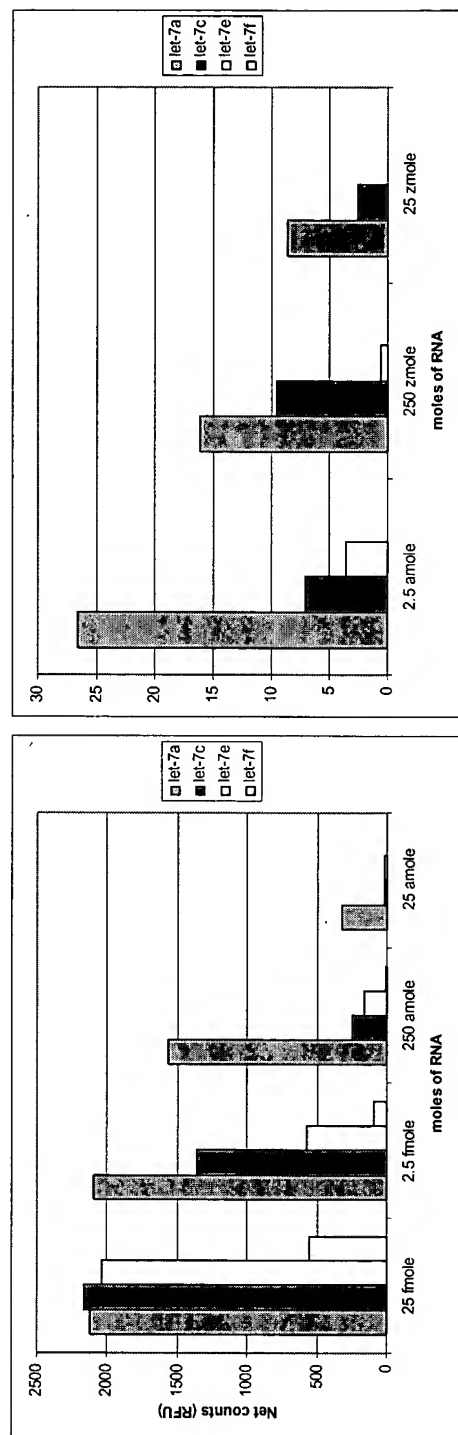
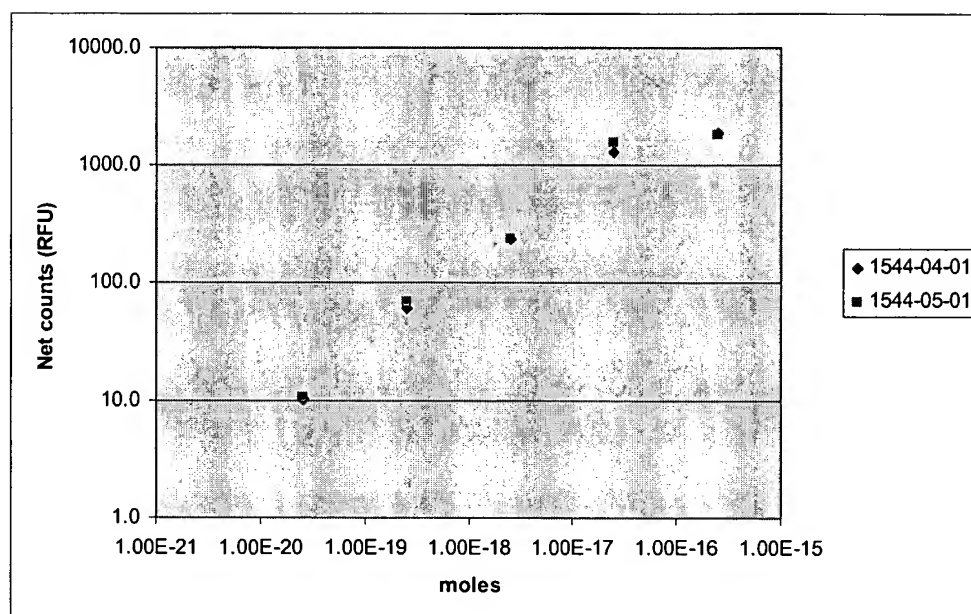


Figure 10
LOD mir-1



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Figure 11
 LOD let-7 (1496-78-01R) using CLEAVASE XII enzyme

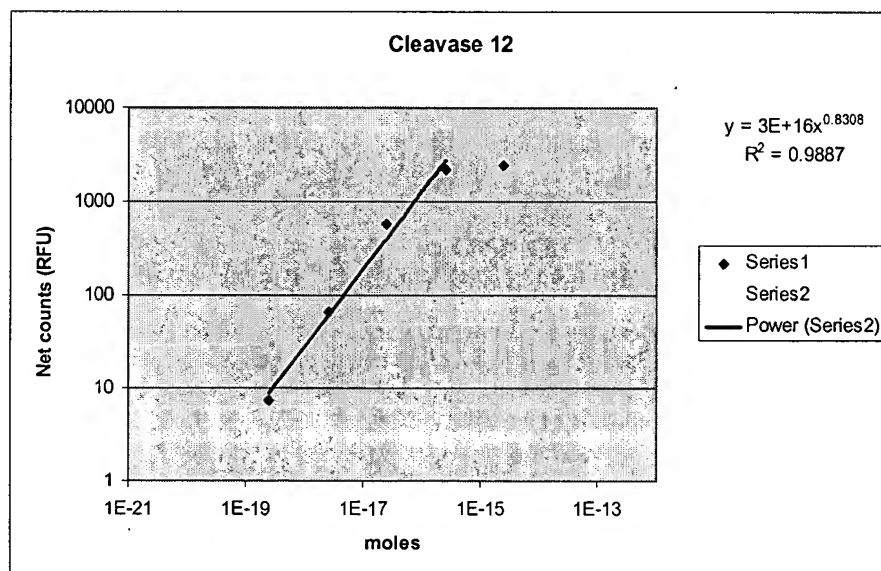
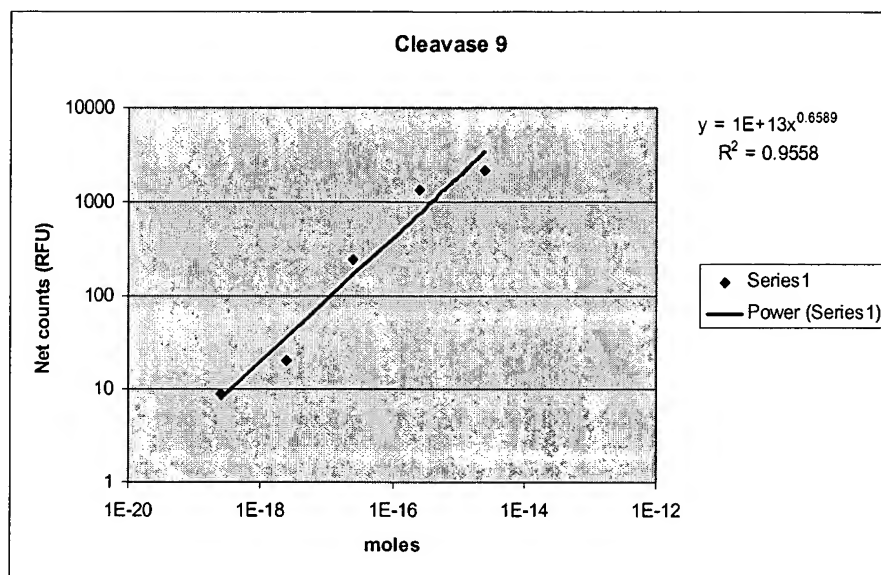


FIGURE 12

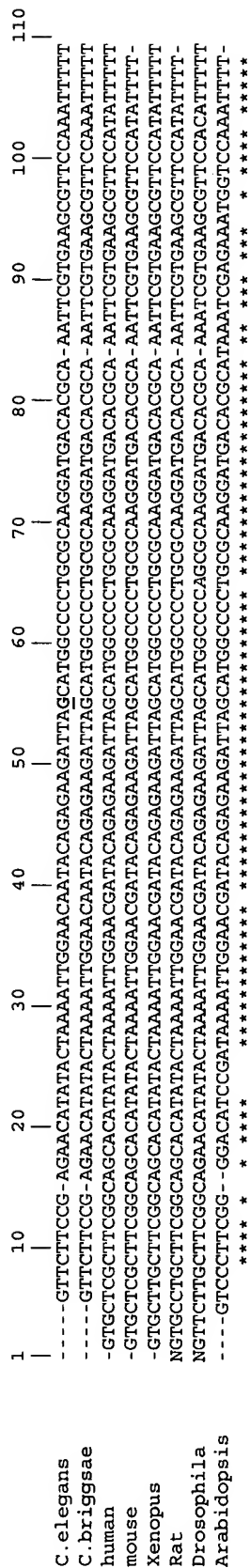


Figure 13

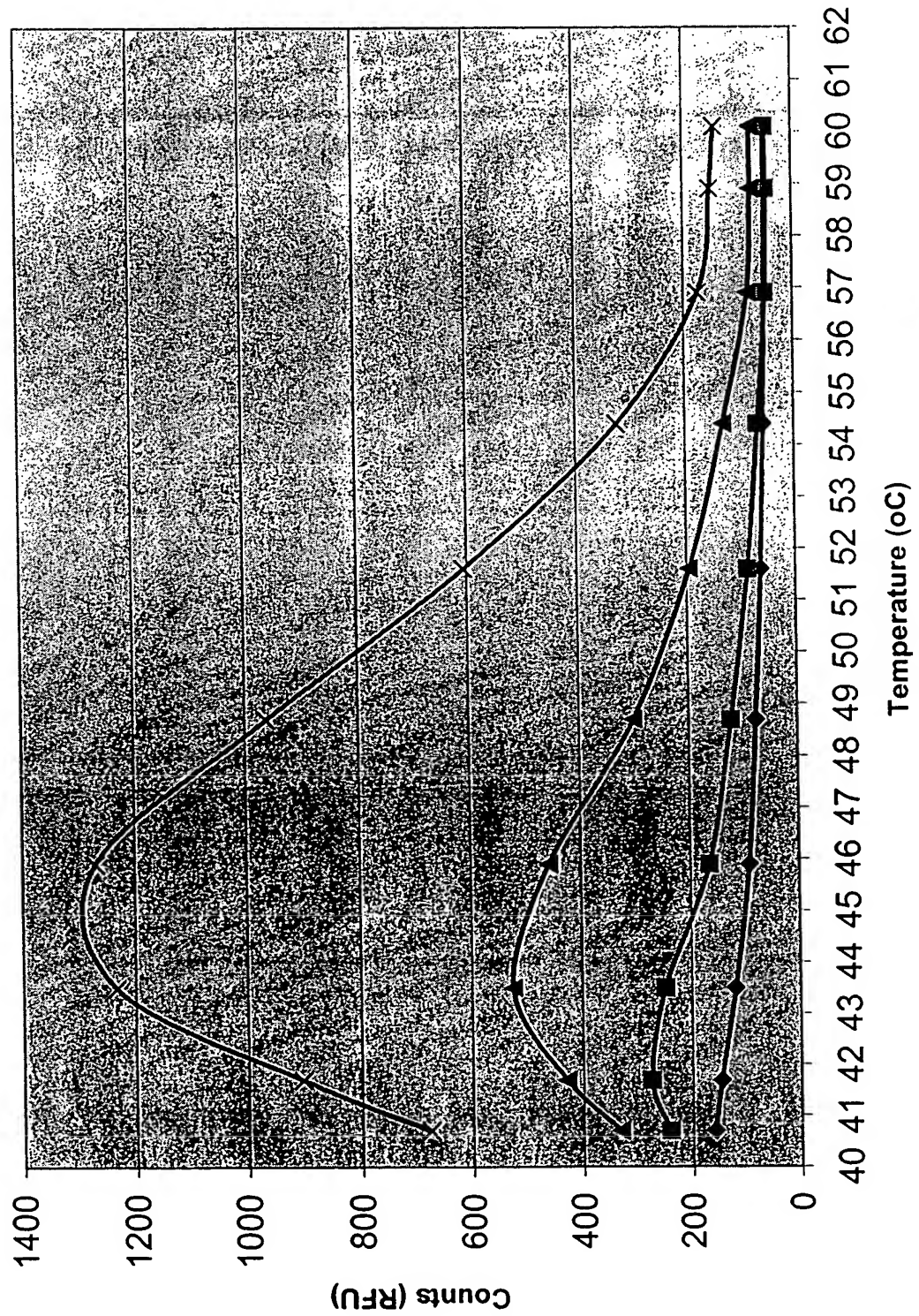


Figure 14

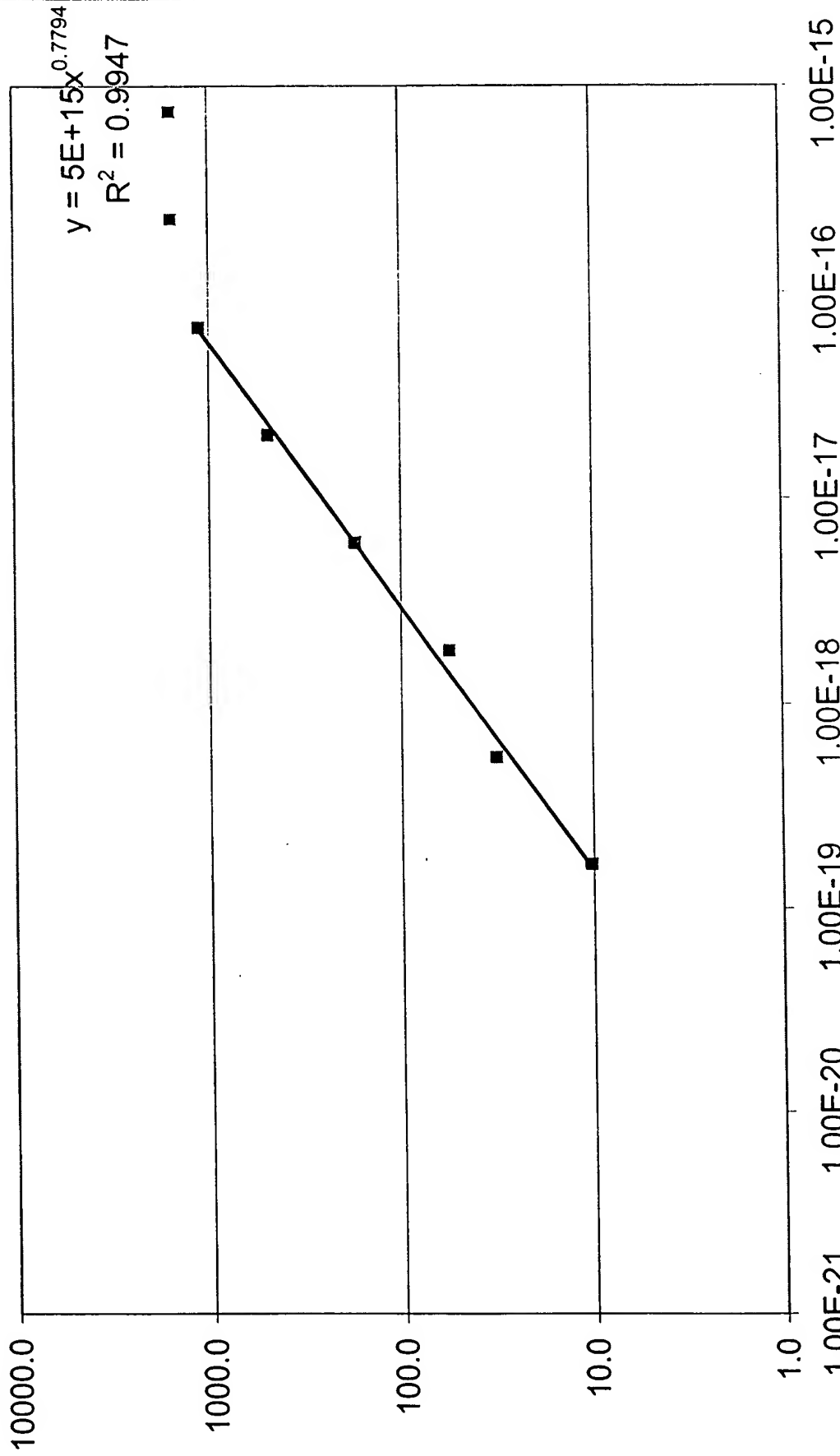


Fig. 15

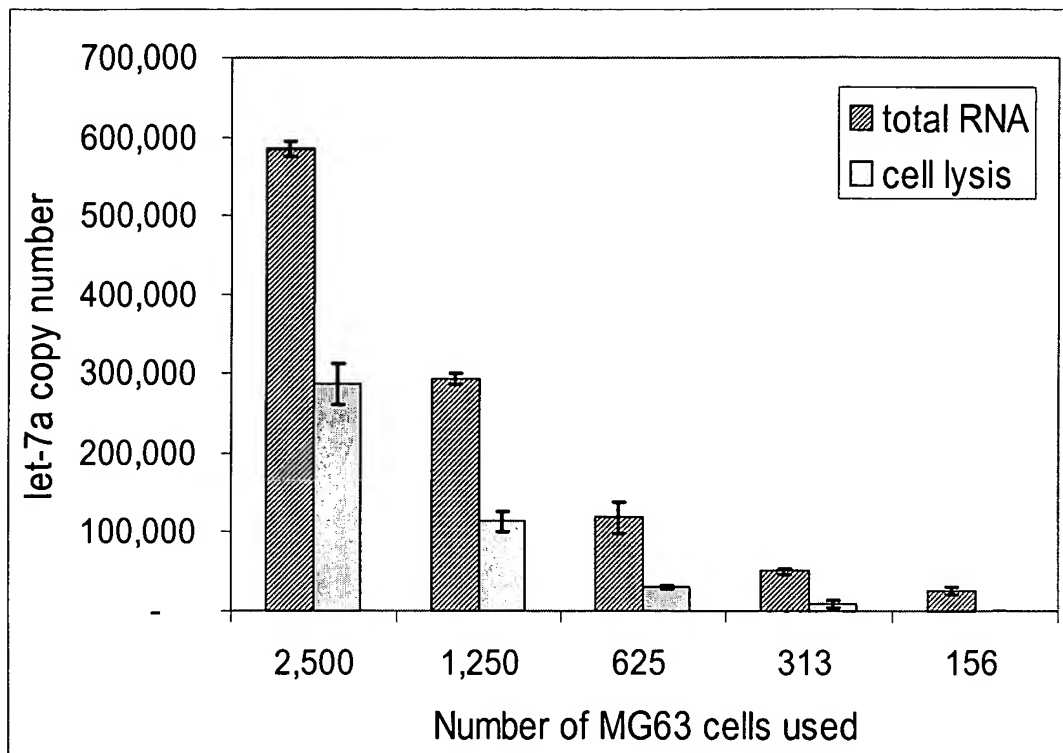
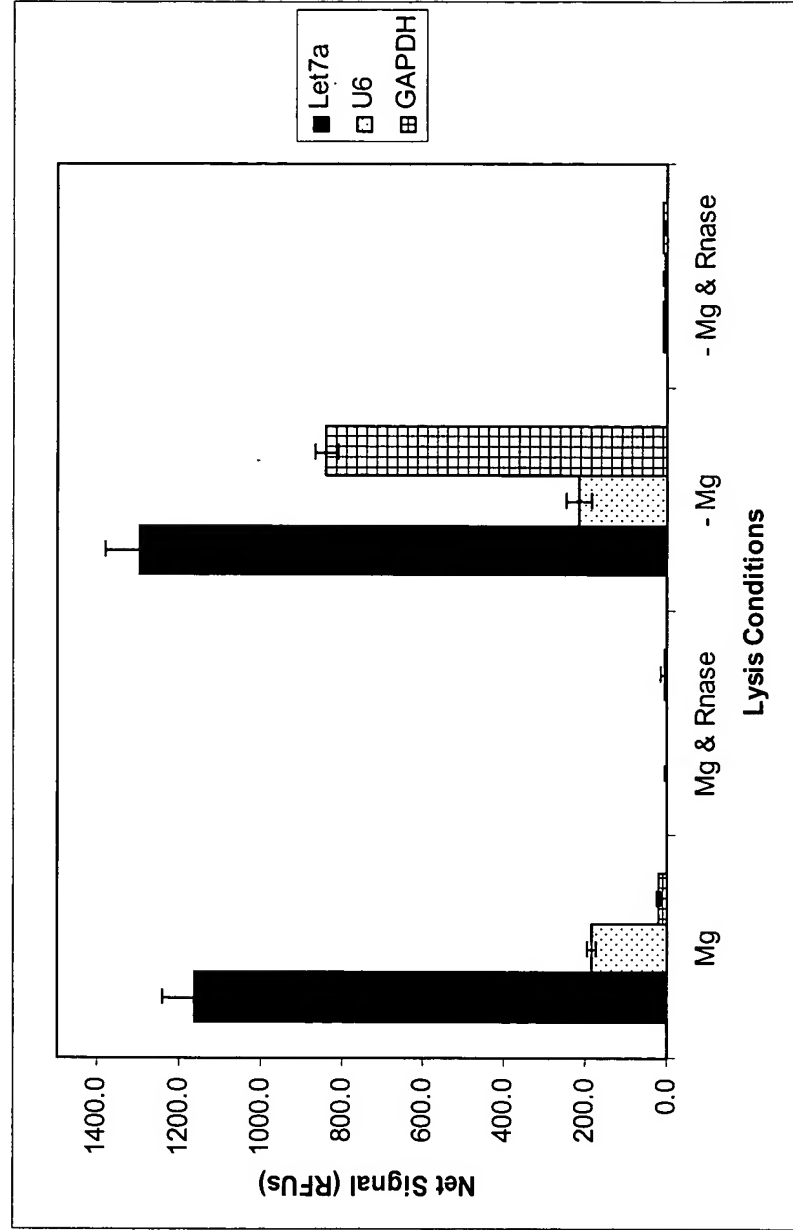
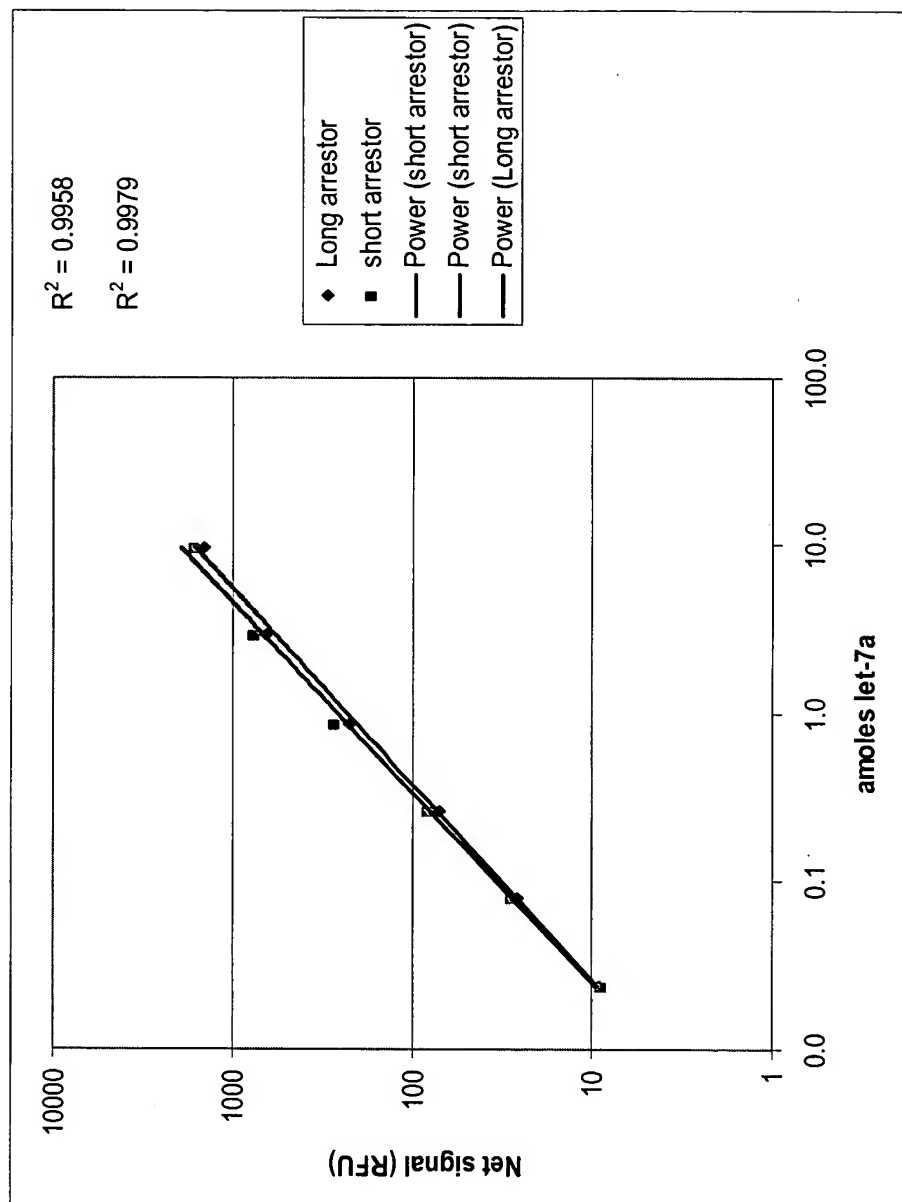


Figure 16



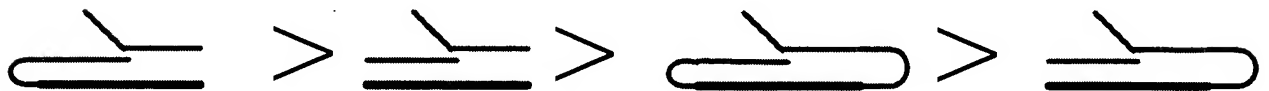
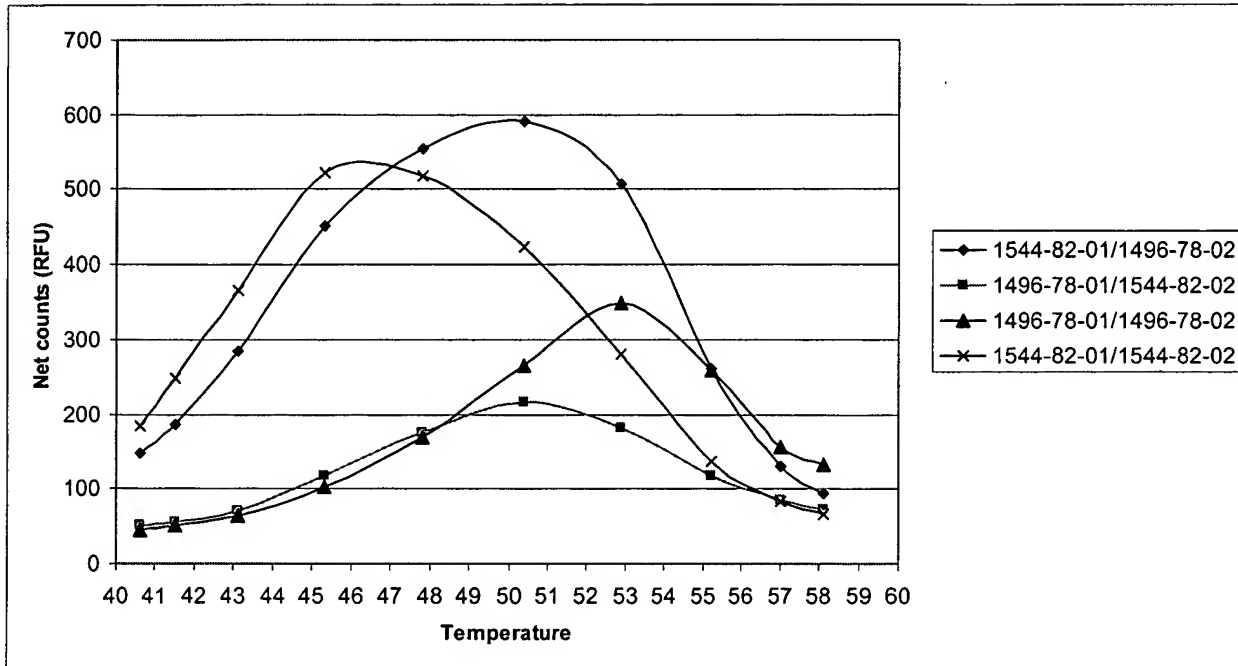
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Figure 17



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FIGURE 18



Normalized
maximum
performance

100%

90%

60%

30%

optimum
temperature
at maximum
performance

50 °C

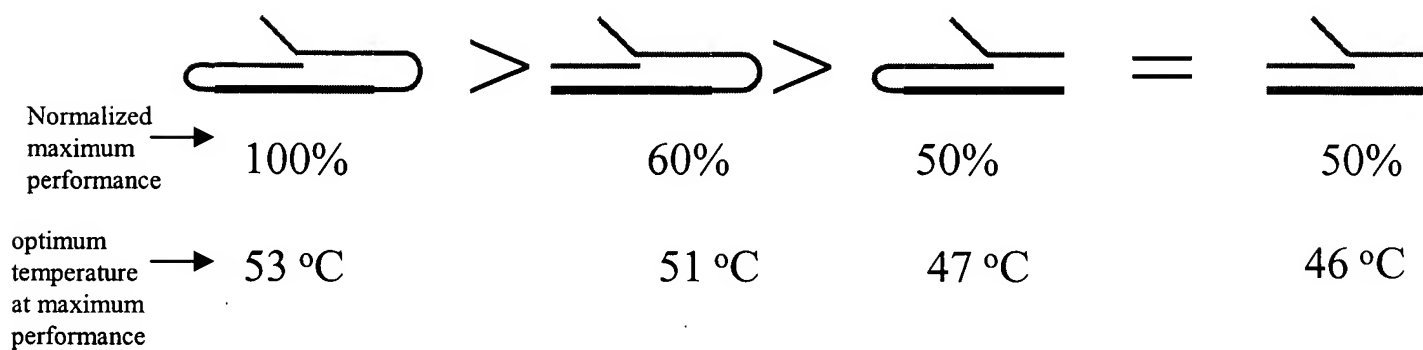
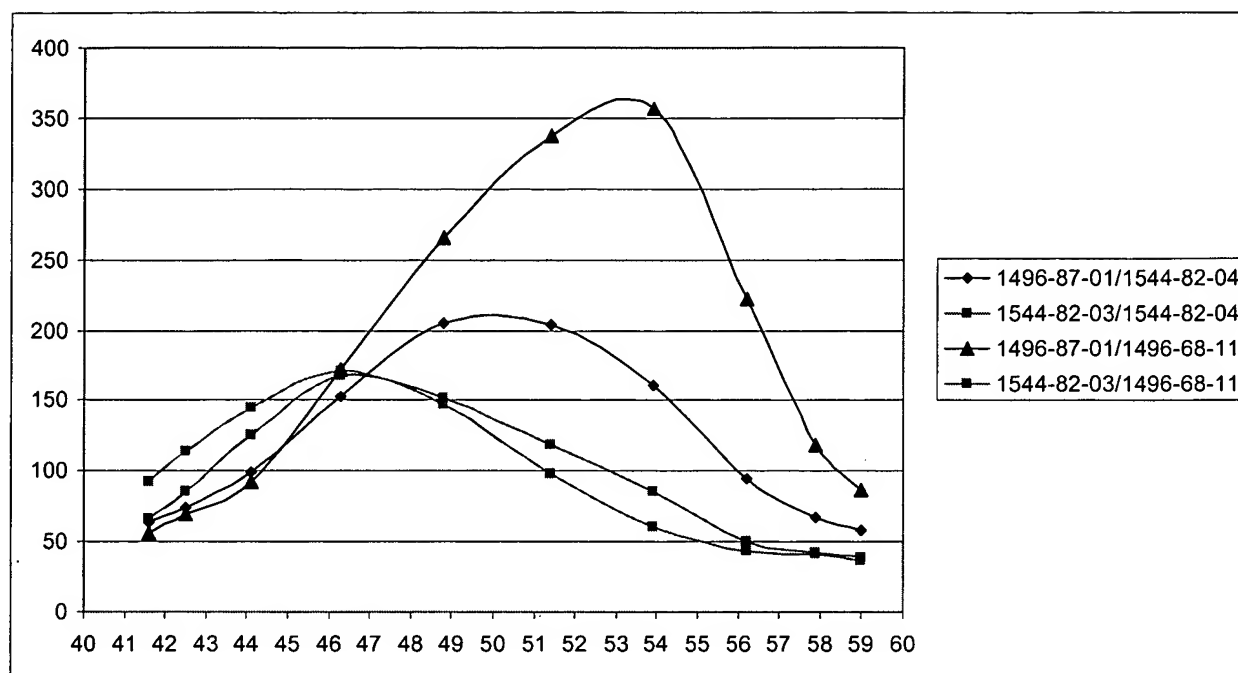
46 °C

53 °C

50 °C

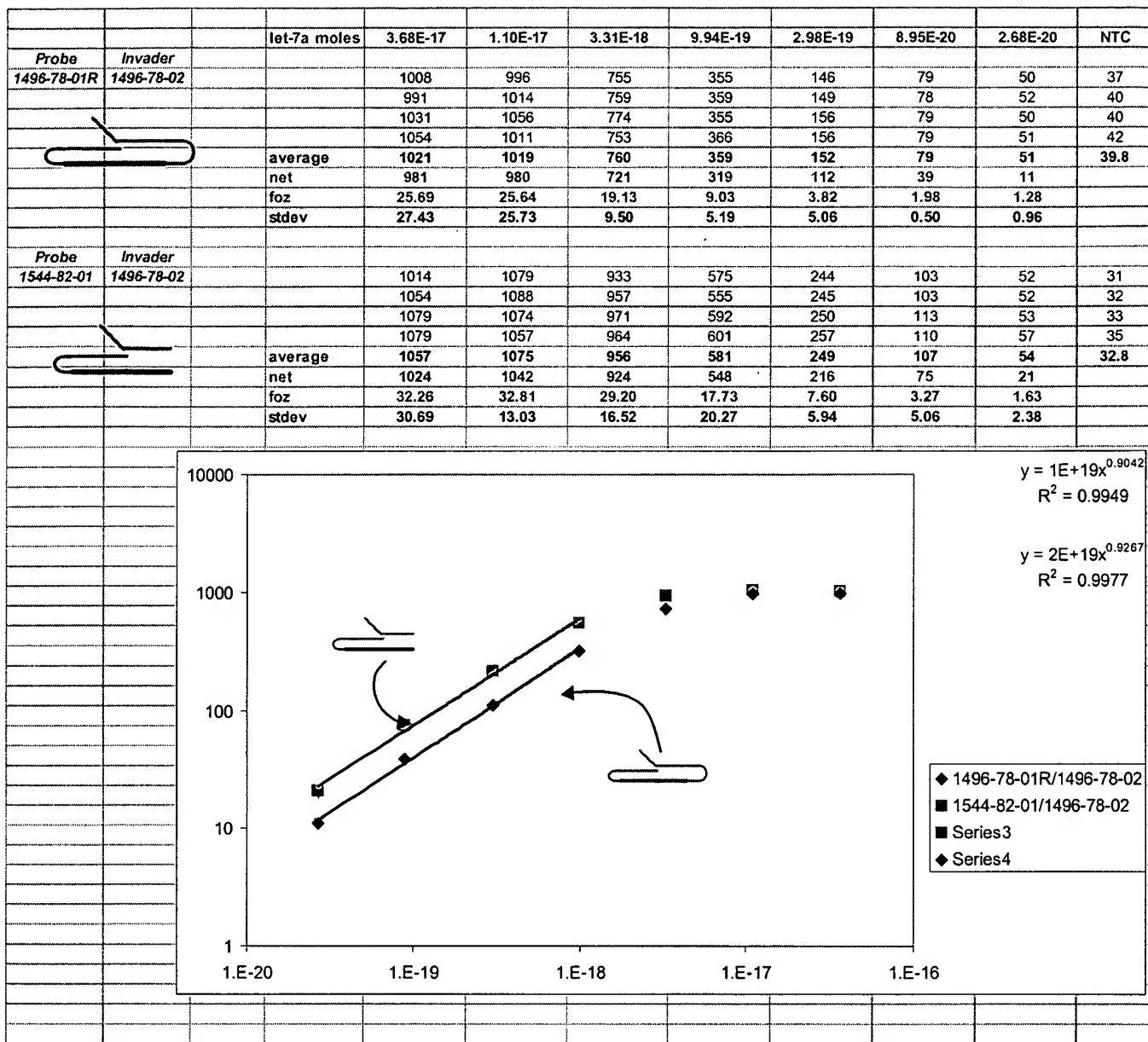
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Fig. 19



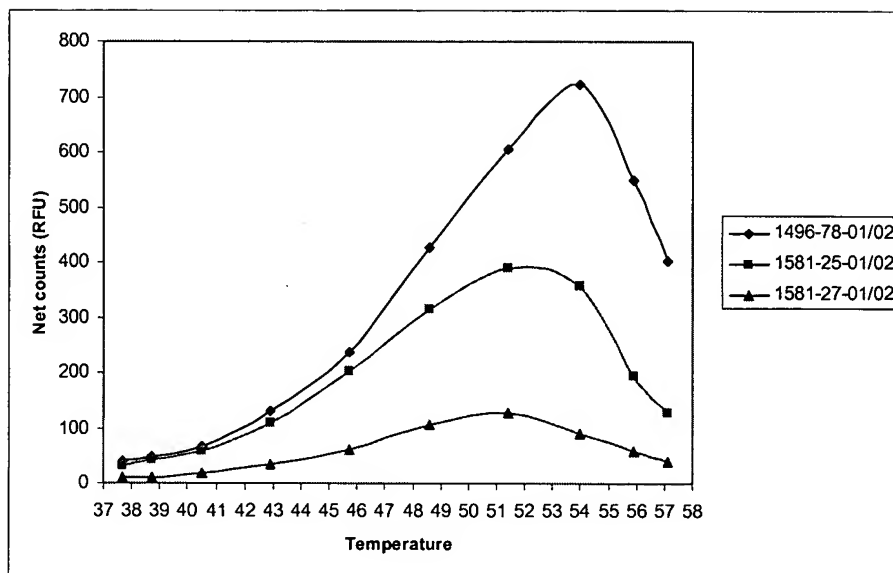
$\frac{\partial \eta}{\partial T}$

Fig. 20



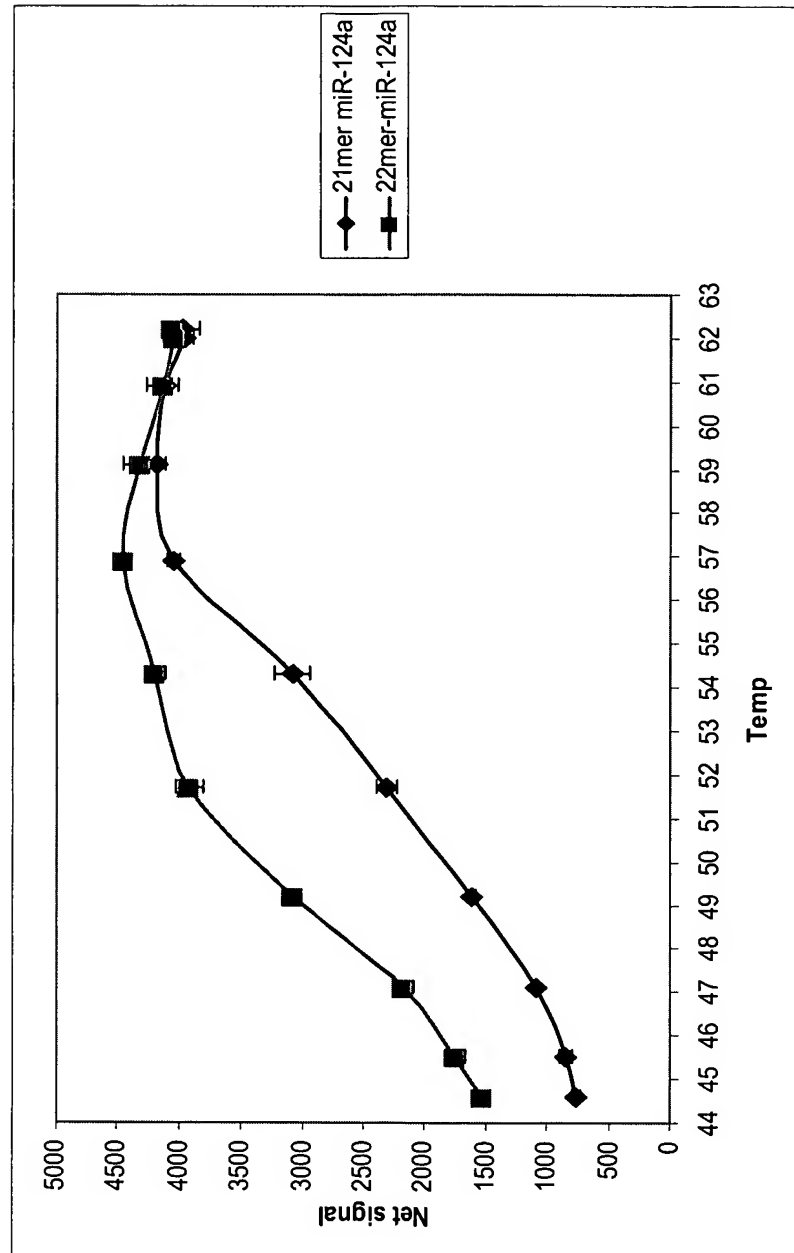
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Figure 21



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Figure 22



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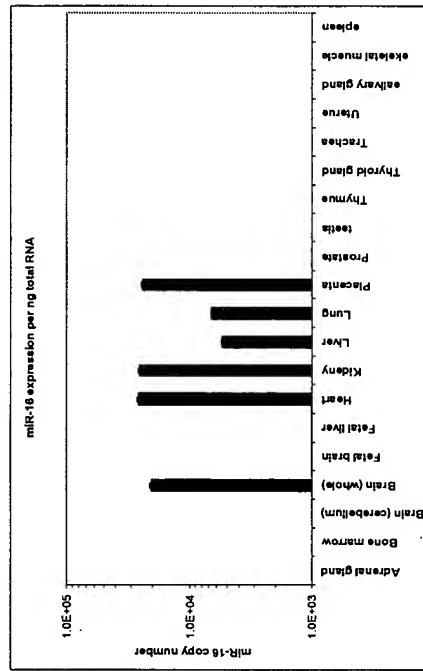
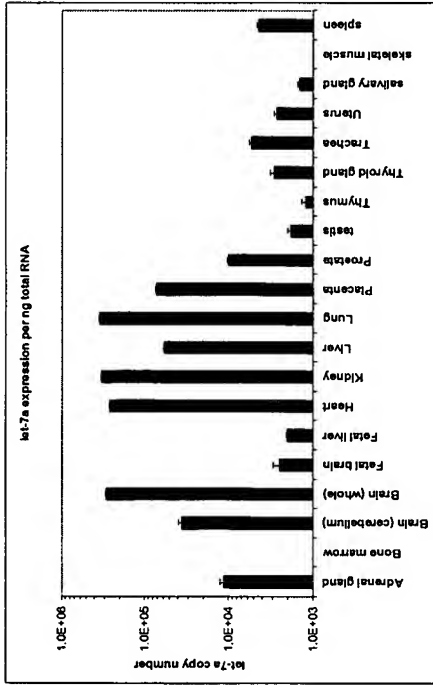
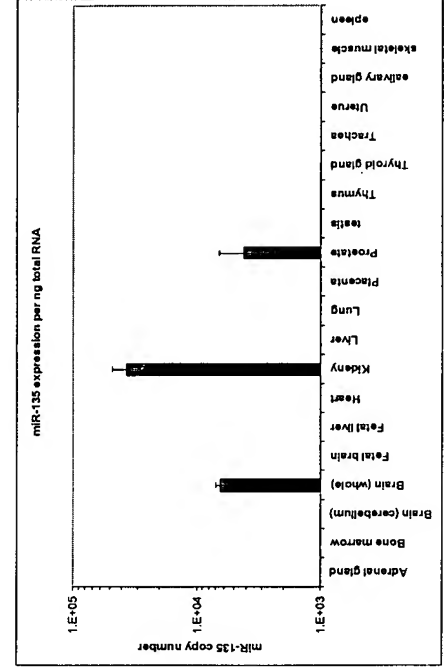
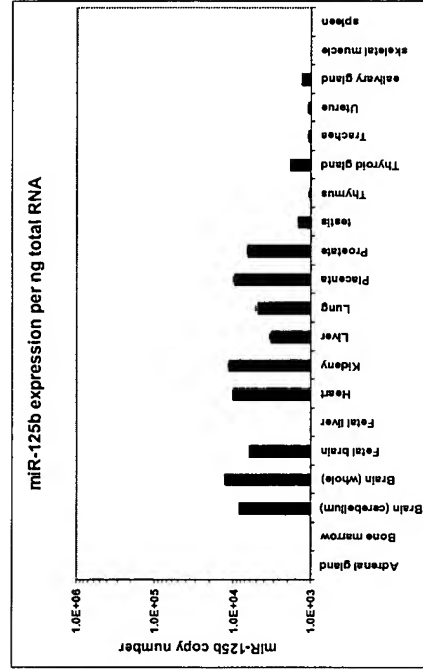
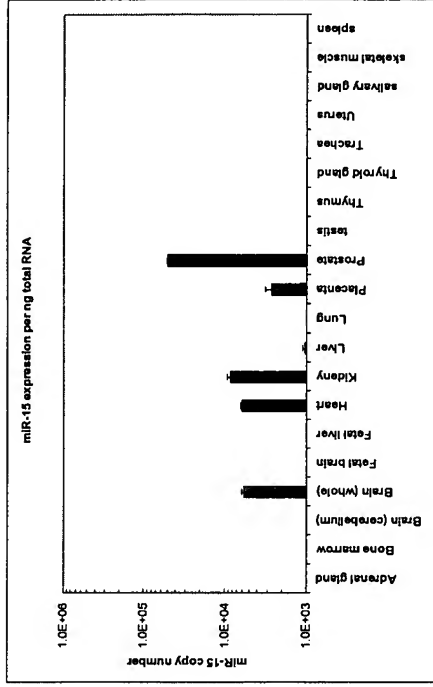


Fig. 23

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Fig. 24

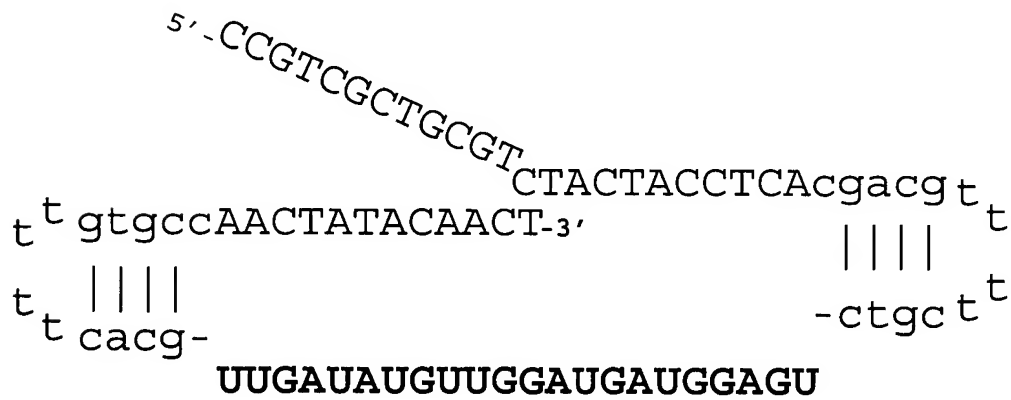
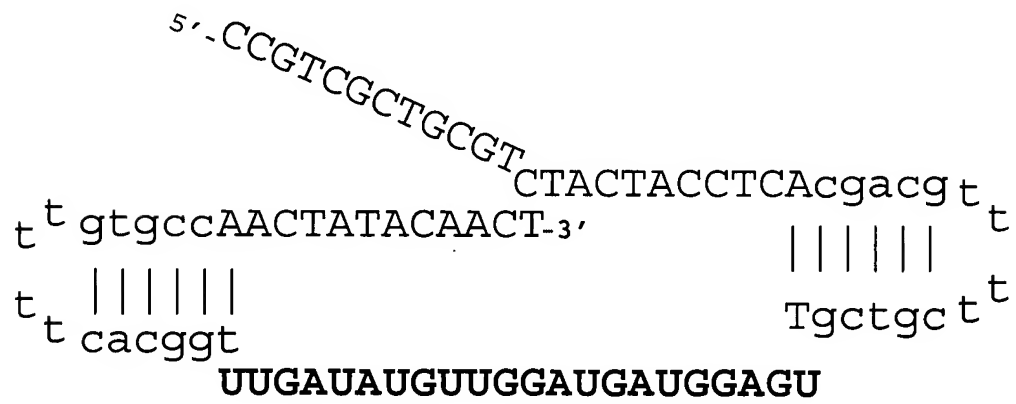
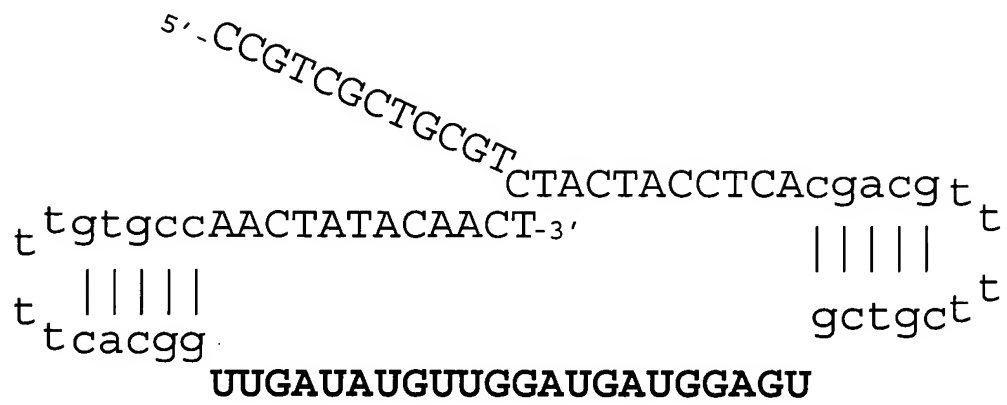


Figure 25

